

DETECTION OF ADULTERATION OF EXTRA VIRGIN OLIVE OIL USING FTIR SPECTRAL DATA ANALYSIS

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ABSTRACT

Determination of authenticity of extra virgin olive oils has become very important in recent years due to the increasing public concerns about possible adulterations with relatively cheap vegetable oils such as sunflower oil, soybean oil, sesame oil, corn oil and refined olive oil. Recent developments in Fourier transform infrared (FT-IR) spectroscopy instrumentation extend the application of this technique to the field of food research, facilitating particularly the studies on edible oils and fats. In this work, FT-IR spectroscopy is used as an effective analytical tool in order to determine extra virgin olive oil adulteration with sunflower, corn and refined olive oils in their binary admixtures in different concentrations (0, 5, 10, 20, 30, 40, 50, 100% - w/w). The spectral region ($1300\text{--}1000\text{ cm}^{-1}$) which contains the IR fingerprints of these vegetable oils was found to be very useful in detecting olive oil adulteration. A band shift observed at 3009 cm^{-1} assigned to the $=\text{C}\text{--}\text{H}$ stretching vibration of the *cis*- double bond, allows the determination of extra virgin olive oil adulteration. The intensities of the spectral bands at 1163 cm^{-1} (assigned to $\text{--C}\text{--}\text{O}$ stretching vibration and CH_2 bending vibration) increase with increasing adulterant concentration. The absorbance ratio ($R_{1118/1097\text{ cm}^{-1}}$) decreased with increasing adulterant concentration. Also, there was a pronounced shift of the peak at 912.78 cm^{-1} (assigned to $\text{--HC}\text{=}\text{CH--}$ *cis*- double bond, bending out of plane) for pure olive oil to higher wave numbers with increasing adulterant concentration.

KEY WORDS: Adulteration; extra virgin olive oil; sunflower oil; corn oil; refined olive oil; FTIR spectroscopy.

INTRODUCTION

Olive oil, used since 4000 B.C. by the Mediterranean populations as a food, drug, and cosmetic, has been the object of numerous epidemiologic, clinical, and experimental studies in the last few decades, confirming its protective action and demonstrating how the intuition of ancient Mediterranean populations now has scientific backing. Because of increased scientific research, the recognition of virgin olive oil's biologic value has notably increased. Currently, we know that in addition to olive oil's preventive action in the physiologic aging process in atherosclerosis and neoplastic development, it exerts protective action toward the skin with topical and dietary use. The Mediterranean diet, which favors the use of fruits, vegetables, bread, pasta, fish, and olive oil, has an effective value in the dermo-cosmetology sector, making ancient Mediterranean customs more comprehensible, such as the use of olive oil on the body after bathing to protect the skin and keep it fresh (**Publio and Marzia 2009**).

The quality of olive oil ranges from the high-quality extra virgin olive oil (EVOO) to the low-quality olive-pomace oil (OPO). EVOO is obtained from the fruit of the olive tree (*Olea europaea* L.) by mechanical press and without application of refining processes. Its acidity cannot be greater than 1%. Due to its high quality, it is considered as the most expensive type of olive oil. For this reason, it is sometimes mislabeled or adulterated. Adulteration involves addition of cheaper oils such as sunflower, soybean, corn, and rapeseed oils (**Guimet et al., 2005**). Also addition of nut oils such as hazelnut and peanut oils was reported too (**Blanch et al., 1998**). Therefore, more rapid and accurate analytical methodologies are being requested for olive oil authentication to be a suitable tool for routine analysis (**Allam and Hamed, 2007**). In this respect, the use of characteristic components such as (E)-5-methyl-hept-2-en-4-one (filbertone), the characteristic flavor compound in hazelnuts, has been proposed as a suitable indicator of the presence of virgin hazelnut oil in olive oil (**Blanch et al., 1998; Guimet et al., 2005; Flores et al., 2006**). However, the usefulness of such compounds to detect adulteration when refined oils are involved is quite difficult.

Another interesting aspect is the possibility of performing rapid and simple analysis for screening procedures and confirmatory processes. In these cases, the advantages of using Solid-Phase Microextraction (SPME) have already been reported (**Arthur and Pawliszyn, 1998; Cercaci et al., 2003; Kanavouras et al., 2005; Flores et al., 2006**). Others described an innovative technique based on X-ray scattering applied to classify complex organic matrices of different vegetable oils. Vegetable oils from corn, canola, soybean, sunflower and olive (extra virgin and others) were analyzed and classifications were obtained using information from the scattered radiation (**Bortoleto et al., 2005**). Among the established methods for the control of authenticity of olive oil come the powerful separation techniques of gas chromatography (**Mannina et al., 1999; Abidi, 2001; Andrikopoulos et al., 2001; Cercaci et al., 2003; Hilali et al., 2007**). High performance liquid chromatography (HPLC) techniques have more recently been developed and coupled with ultraviolet and mass spectroscopy (**Cañabate-Díaz et al., 2007**). By all techniques, certain compounds contained in oils are detected, analyzed and used for detecting the adulteration of virgin olive oils.

Spectroscopy was employed successfully in this situation. Nuclear magnetic resonance (NMR) spectroscopy is applied successfully to the analysis of mixtures of virgin olive oil with oils of different botanical origin (**Fauhl et al., 2000; Vlahov, 2006; Christophoridou and Dais, 2009**). Spectrofluorometric methods were also reported for detecting the adulteration of olive oil (**Guimet et al., 2004; Guimet et al., 2005; Guimet et al., 2006**). Infrared (IR) spectroscopy in the visible and near infrared (NIR) regions showed an excellent potential as an analytical method discriminating between extra virgin olive and seed oils (**Downey et al., 2002; Armenta et al., 2007, Özdemir and Öztürk, 2007**).

More recently, FTIR spectroscopy has been shown to be the most powerful tool for identification and authentication of edible oils (**Tay et al., 2002; Allam and Hamed, 2007; Sherazi et al., 2009**).

The present work aimed at the applications of FTIR spectroscopy as a rapid, cheap nondestructive, authenticity measuring tool to assess the adulteration of extra virgin olive oil with other edible oils such as corn, sunflower and refined olive oils. Library searching in the FTIR region is a well established and powerful way which was used in comparison and matching of measured spectra.

MATERIALS AND METHODS

Materials:

Extra virgin olive oil was extracted from fresh olive fruits by Oliomio-machine then filtered and kept in brown glass bottles at -5°C . Refined olive oil, sunflower oil and corn oil samples were purchased locally. According to their labels, all the oils used in adulteration were additive-free, refined, bleached and deodorized (RBD). Solvents and chemicals used were all of analytical grades.

Methods:

Different admixtures of various concentrations (0, 5, 10, 20, 30, 40, 50, 100%) of the above used edible oils were prepared (w/w) as binary mixtures with extra virgin olive oil.

Instrumentation and Spectral Data Acquisition:

A Jasco Spectrum FT-IR Plus 460 Spectrophotometer (Japan) equipped with a deuterated triglycerine sulphate (DTGS) detector was used to obtain FT-IR spectra. $5\mu\text{l}$ of the sample was pressed between two well-polished KBr disks (liquid cell) creating a thin film. Each sample undergoes 64 scans which are accumulated in one measurement to acquire a sufficient signal-to-noise ratio. Samples were scanned between 4000 and 400cm^{-1} with a nominal resolution of 4cm^{-1} and the data interval was 1cm^{-1} . (Allam and Hamed, 2007).

RESULTS AND DISCUSSION

Figure (1) shows the typical FTIR spectra of the edible oils used in this study. These oils include 100% olive, 100% sunflower, 100% corn and 100% refined olive oils. As shown from this figure, it is difficult to differentiate between these oils by just visual examination of their whole spectrum. However, a careful investigation of the fingerprint region specifically the spectral region ($1300\text{-}1000\text{ cm}^{-1}$), revealed that there are visual differences in the absorption intensity at 1163 cm^{-1} (assigned to -C-O stretching and -CH_2 bending) and at 1118 cm^{-1} (assigned to -C-O stretching). Guillen and Cabo, 1999 mentioned that adulterant oils exhibit some intensity in the band around $913\text{-}914\text{ cm}^{-1}$, whereas intensity and position of band is different for olive oil showing very low or no intensity. This band was employed to detect and quantify adulteration.

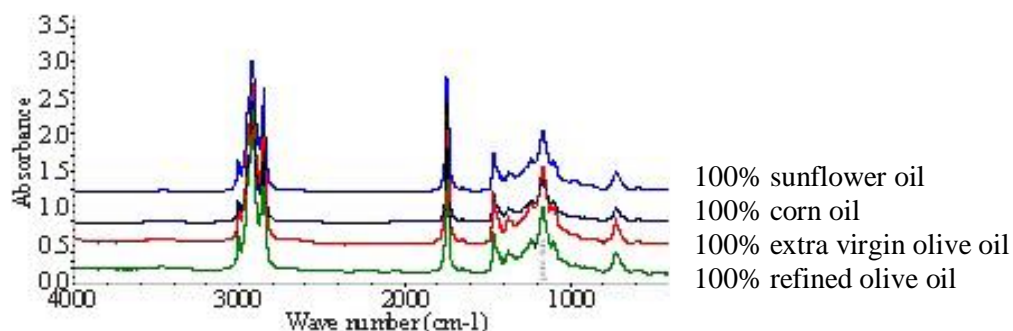


Fig. 1. Typical FTIR Absorption spectra (4000-400 cm^{-1}) of pure oils (sunflower, corn, extra virgin olive and refined olive oils)

From the spectral data comparison between pure oils, there exist notable differences in the band around 3005.52 cm^{-1} assigned to C-H stretching vibration of *cis*- double bond ($=\text{CH}$). The oil composition affects the exact position of the band and yields shifts when the proportion of fatty acid changes. Sunflower and corn oils showed exactly identical absorbance at 3008.41 cm^{-1} , compared to extra virgin olive and refined olive oils which had exactly identical absorbance at 3005.52 cm^{-1} .

Figures 2,3, and 4 display these spectral fingerprints in which one can visualize easily that the intensity of the absorption peak at 1163 cm^{-1} decreases by increasing the concentration of sunflower, corn, and refined olive oils , respectively.

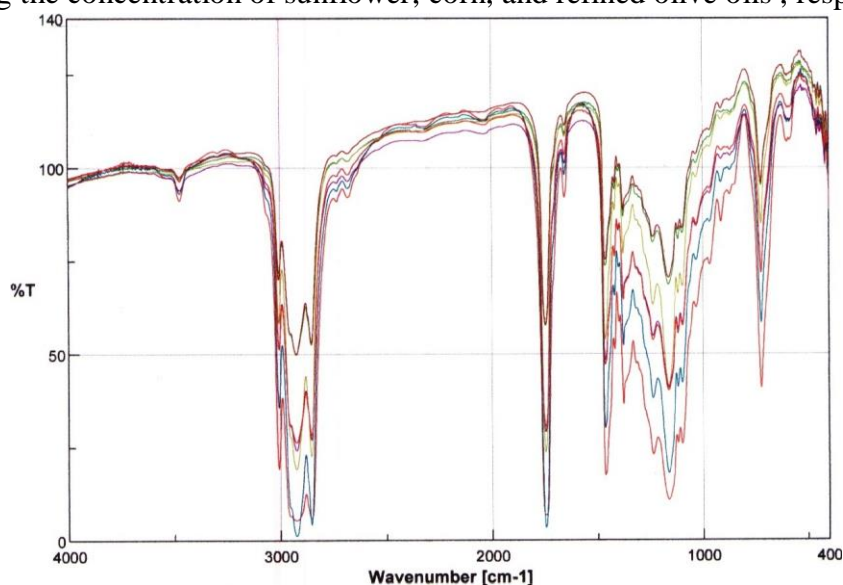


Fig. 2. Typical FTIR spectra of pure olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of sunflower oil (bottom).

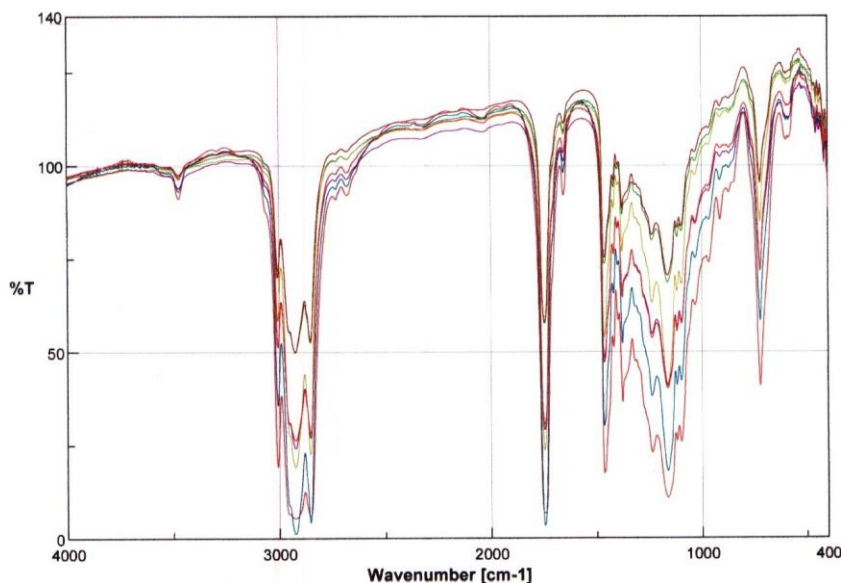


Fig. 3. Typical FTIR spectra of pure olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of corn oil (bottom).

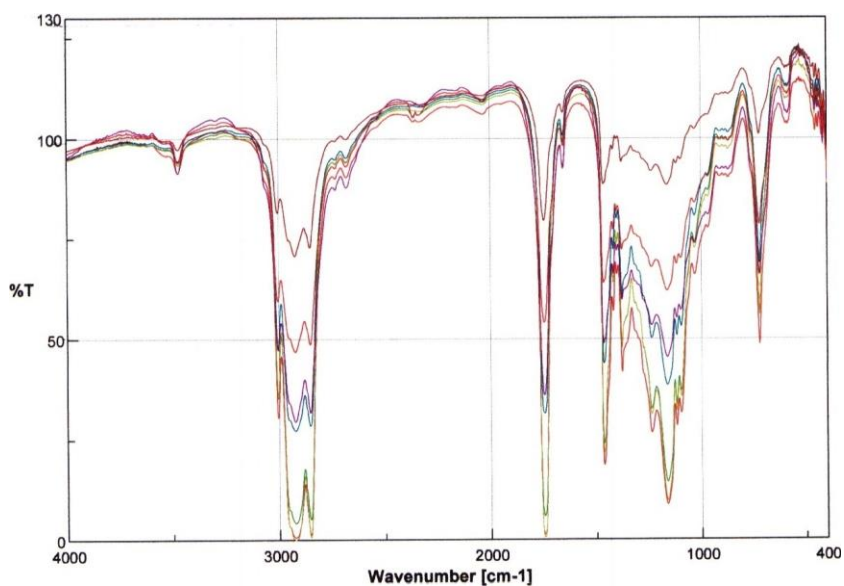


Fig. 4. Typical FTIR spectra of pure olive oil (top) mixed with different ratios (0, 5, 10, 20, 30, 40, 50 and downwards till 100%) of refined olive oil (bottom).

Table 1. Absorption Intensity (at 1163 cm⁻¹) of EVOO in binary admixtures with different adulterant oils (SO, CO and ROO) at various concentrations.

| Adulterant oil % | Absorption Intensity at 1163.83 cm ⁻¹ | | |
|---------------------|--------------------------------------------------|-------------------------------------|--------------------------------------|
| | EVOO ¹ + SO ² | EVOO ¹ + CO ³ | EVOO ¹ + ROO ⁴ |
| 0 (EVOO) | 0.416 | 0.416 | 0.416 |
| 5 | 0.445 | 0.452 | 0.447 |
| 10 | 0.455 | 0.463 | 0.492 |
| 20 | 0.509 | 0.503 | 0.553 |
| 30 | 0.653 | 0.587 | 0.653 |
| 40 | 0.708 | 0.654 | 0.741 |
| 50 | 0.907 | 0.743 | 0.907 |
| 100 | 1.329 | 0.959 | 1.054 |

Table 2. Shift of Absorption Peak (at 912.78 cm⁻¹) of EVOO in binary admixtures with different adulterant oils (SO, CO and ROO) at various concentrations.

| Adulterant oil % | Shift of absorption peak at 912.780 cm ⁻¹ | | |
|---------------------|------------------------------------------------------|-------------------------------------|--------------------------------------|
| | EVOO ¹ + SO ² | EVOO ¹ + CO ³ | EVOO ¹ + ROO ⁴ |
| 0 (EVOO) | 912.780 | 912.780 | 912.780 |
| 5 | 912.780 | 912.780 | 912.780 |
| 10 | 913.129 | 913.129 | 912.780 |
| 20 | 913.129 | 913.129 | 913.129 |
| 30 | 913.129 | 914.093 | 913.129 |
| 40 | 913.129 | 914.093 | 913.129 |
| 50 | 914.093 | 914.093 | 914.093 |
| 100 | 914.093 | 914.093 | 914.093 |

Table 3. Absorbance Ratios (R1118/1097 cm⁻¹) of EVOO in binary admixtures with different adulterant oils (SO, CO and ROO) at various concentrations.

| Adulterant oil % | R1118/1097 cm ⁻¹ | | |
|---------------------|-------------------------------------|-------------------------------------|--------------------------------------|
| | EVOO ¹ + SO ² | EVOO ¹ + CO ³ | EVOO ¹ + ROO ⁴ |
| 0 (EVOO) | 1.090 | 1.090 | 1.090 |
| 5 | 1.054 | 1.080 | 1.087 |
| 10 | 1.042 | 1.066 | 1.080 |
| 20 | 1.037 | 1.058 | 1.079 |
| 30 | 1.034 | 1.042 | 1.077 |
| 40 | 1.027 | 1.033 | 1.069 |
| 50 | 1.016 | 1.031 | 1.065 |
| 100 | 0.939 | 0.992 | 1.030 |

Abbreviations: 1: Extra virgin olive oil, 2: Sunflower oil, 3: Corn oil, 4: Refined olive oil

In addition to the above mentioned changes, there is another important measure noticed from the spectral data, this is the shift in position of the peak at 912.78 cm^{-1} (assigned to $-\text{HC}=\text{CH}-$, *cis*-, bending out of plane) for extra virgin olive oil to higher wave numbers: 914.093 , 914.093 , and 914.093 cm^{-1} with the increase of concentration of sunflower, corn, and refined olive oils, respectively as shown in table 2.

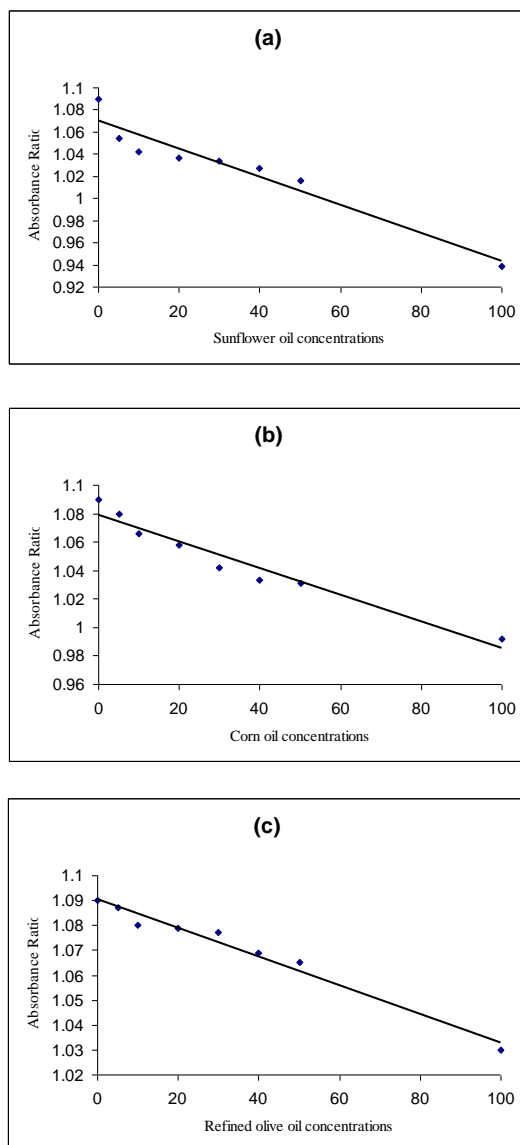


Fig. 5. The relationship between changes in Absorbance Ratio ($R_{1118/1097}\text{ cm}^{-1}$) and concentrations of: sunflower oil (a), corn oil (b) and refined olive oil (c)

From tables (1 and 3), the intensities of the spectral bands at 1163 cm^{-1} (assigned to -C-O stretching vibration and CH_2 bending vibration) increase with increasing adulterant concentration. The absorbance ratio ($\text{R}_{1118/1097\text{ cm}^{-1}}$) decreased with increasing adulterant concentration.

From the above three relations represented by figure 5, it is possible to determine the adulterant concentration of sunflower, corn, and refined olive oils, respectively by simply calculating the absorbance ratio ($\text{R}_{1118/1097\text{ cm}^{-1}}$) then concluding its value from the linear relationship plot.

CONCLUSION

It is concluded that the adulteration of extra virgin olive oil can be monitored by using FTIR spectroscopy via measuring the changes in absorbance ratio ($\text{R}_{1118/1097\text{ cm}^{-1}}$) of absorbance intensity and shift of peak at 912.78 cm^{-1} to higher wavenumber. This technique has been shown to be a powerful tool to detect the concentration of adulterant oil at low percentage as 5%.

REFERENCES

- Abidi, S.L. (2001).** Chromatographic analysis of plant sterols in foods and vegetable oils. **J. Chromatography A**, **935**: 173–201.
- Allam, M.A., and S.F. Hamed (2007).** Application of FTIR spectroscopy in the assessment of olive oil adulteration. **J. Applied Sciences Research**, **3**: (2), 102-108.
- Andrikopoulos, N.K., I.G. Giannakis, and V. Tzamtzis (2001).** Analysis of olive oil and seed oil triglycerides by capillary gas chromatography as a tool for the detection of the adulteration of olive oil. **J. Chromatographic Science** 137-145.
- Armenta, S., S. Garrigues, and M. De La Guardia (2007).** Determination of edible oil parameters by near infrared spectrometry. **Analytica Chimica Acta** **596**: 330–337.
- Arthur, C.L., and J. Pawliszyn (1998).** Solid phase microextraction with thermal desorption using fused silica optical fibers. **Analytical Chemistry** **62**: 2145-2148.

Blanch, G.P., M.M. Caja, M.L. Ruiz del Castillo, and M. Herraiz (1998). Comparison of different methods for the evaluation of the authenticity of olive oil and hazelnut oil. **J. Agric. Food Chem. 46: 3153-3157.**

Bortoleto, G.G., L.C.M. Pataca, and M.I.M.S. Bueno (2005). A new application of X-ray scattering using principal component analysis – classification of vegetable oils. **Analytica Chimica Acta 539: 283–287.**

Cañabate-Díaz, B., A.S. Carretero, A. Fernández-Gutiérrez, A.B. Vega, A.G. Frenich, J.L.M. Vidal, and J.D. Martos (2007). Separation and determination of sterols in olive oil by HPLC-MS. **Food Chemistry 102: 593–598.**

Cercaci, L., M.T. Rodriguez-Estrada, and G. Lercker (2003). Solid phase extraction, thin layer chromatography and gas chromatography method for the detection of hazelnut oil in olive oils by determination of esterified sterols. **J. Chromatography A, 985: 211–220.**

Christophoridou, S., and P. Dais (2009). Detection and quantification of phenolic compounds in olive oil by high resolution ^1H nuclear magnetic resonance spectroscopy. **Analytica Chimica Acta 633: 283-292.**

Downey, G., P. McIntyre, and A.N. Davies (2002). Detecting and quantifying sunflower oil adulteration in extra virgin olive oils from the eastern Mediterranean by visible and near-infrared spectroscopy. **J. Agric. Food Chem., 50: 5520 -5525.**

Fauhl, C., F. Reniero, and C. Guillou (2000). ^1H NMR as a tool for the analysis of mixtures of virgin olive oil with oils of different botanical origin. **Magnetic Resonance in Chemistry 38: 436-443.**

Flores, G., M.L. Ruiz del Castillo, G.P. Blanch, and M. Herraiz (2006). Detection of the adulteration of olive oils by solid phase microextraction and multidimensional gas chromatography. **Food Chemistry 97: 336-342.**

Guillén, M.D., and N. Cabo (1997). Infrared spectroscopy in the study of edible oils and fats. **J. Sci. Food Garic., 75: 1–11.**

Guillén, M. D., and N. Cabo (1999). Usefulness of the frequencies of some Fourier transform infrared spectroscopic bands for evaluating the composition of edible oil mixtures. **Fett/Lipid, 101: 71–76.**

Guimet, F., J. Ferré, and R. Boqué (2005). Rapid detection of olive–pomace oil adulteration in extra virgin olive oils from the protected denomination of origin “Siurana” using excitation–emission fluorescence spectroscopy and three-way methods of analysis. **Analytica Chimica Acta 544: 143–152.**

Guimet, F., R. Boqué, and J. Ferré (2006). Application of non-negative matrix factorization combined with Fisher's linear discriminant analysis for classification of olive oil excitation–emission fluorescence spectra. **Chemometrics and Intelligent Laboratory Systems 81: 94 – 106.**

Guimet, F., J. Ferré, R. Boqué, and F.X. Rius (2004). Application of unfold principal component analysis and parallel factor analysis to the exploratory analysis of olive oils by means of excitation-emission matrix fluorescence spectroscopy. **Analytica Chimica Acta 515: 75-85.**

Hilali, M., Z. Charrouf, A. Soulhi, L. Hachimi, and D. Guillaume (2007). Detection of aragan oil adulteration using quantitative campesterol GC-analysis. **J. Am. Oil Chem. Soc. 84: 761-764.**

Kanavouras, A., A. Kiritsakis, and R.J. Hernandez (2005). Comparative study on volatile analysis of extra virgin olive oil by dynamic headspace and solid phase microextraction. **Food Chemistry 90: 69-79.**

Mannina, L., M. Patumi, P. Fiordiponti, M.C. Emanuele, and A.L. Segre (1999). Olive and hazelnut oils: a study by high-field ^1H NMR and gas chromatography. **Italian J. Food Science 11: 139-149.**

Özdemir, D., and B. Öztürk (2007). Near-infrared spectroscopic determination of olive oil adulteration with sunflower and corn oils. **J. Food and Drug Analysis 15: 40-47.**

Publio V. and V. Marzia (2009). Virgin olive oil as a fundamental nutritional component and skin protector. **Clinics in Dermatology 27: 159–165.**

Sherazi, S.T.H., A. Kandhro, S.A. Mahesar, M.I. Bhanger, M.Y. Talpur, and S. Arain (2009). Application of transmission FT-IR spectroscopy for the *trans* fat determination in the industrially processed edible oil. **Food Chemistry 114: 323-327.**

Tay, A., R.K. Singh, S.S. Krishnan, and J.P. Gore (2002). Authentication of olive oil adulterated with vegetable oils using Fourier transform infrared spectroscopy. **Lebensm.-Wiss. u.-Technol. 35: 99–103.**

Vlahov, G. (2006). ^{13}C nuclear magnetic resonance spectroscopy to determine olive oil grades. **Analytica Chimica Acta 577: 281–287.**

كشف الغش في زيت الزيتون البكر الممتاز بتحليل الطيف بمنطقة الأشعة تحت الحمراء

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لقد زادت أهمية الكشف عن الغش في زيت الزيتون البكر الممتاز بتزايد اهتمام الرأي العام بأساليب غش زيت الزيتون بخلطه بالزيوت الأقل جودة وثمناً مثل زيوت عباد الشمس، فول الصويا، السمسم، الذرة، البندق أو بخلطه بزيوت الزيتون الأقل جودة، وذلك بصفة خاصة بعد الاكتشافات الطبية التي لا تحصى والتي أثبتت فوائد زيت الزيتون.

ولقد ساعد التطور المستمر في طرق التحليل الطيفي بالمنطقة تحت الحمراء في مجال بحوث الأغذية على تيسير طرق كشف الغش في زيت الزيتون. وفي هذا البحث يتم استعراض البيانات التحليلية للأشعة تحت الحمراء لكشف الغش في زيت الزيتون الذي تم خلطه بتركيزات مختلفة من الزيوت الشائع استخدامها في الغش (عباد الشمس ، الذرة، الزيتون المكرر) بعمل مخاليط ثنائية من زيوت الغش مع زيت الزيتون البكر (0، 5، 10، 20، 30، 40، 50، 100% - وزن/وزن).

ولقد تم تسجيل بيانات التحليل الطيفي في منطقة الأشعة تحت الحمراء في المدى من 4000-400 سم⁻¹. وكانت النتائج ممتازة حيث لوحظ وجود انحراف طيفي عند القيمة 3009 سم⁻¹ (رابطة CH_2 = المطاطية الاهتزاز الزوجية في الوضع *cis*-) وكانت موجودة في مخاليط الغش وغير موجودة في زيت الزيتون البكر مما يساعد على اكتشاف الغش.

كذلك ظهرت نتائج ممتازة في المدى الطيفي 1300 – 1000 سم⁻¹ والتي اعتبرت كبصمة الإصبع في الزيوت، حيث أظهرت القيمة 1163 سم⁻¹ (رابطة C-O - المطاطية الاهتزاز ورابطة CH_2 الإلتوائية الاهتزاز) زيادة في شدة الامتصاص عند هذه النقطة بازدياد معدلات الغش في زيت الزيتون.

كانت نسبة الامتصاص ($R = 1097/1118$ سم⁻¹) مفيدة جداً حيث انخفضت هذه النسبة بتزايد نسبة الغش.

كما ظهرت إزاحة واضحة لمنحنى الرابطة (HC=CH -) عند القيمة 912 سم⁻¹ نتيجة للالتواء الحادث في الرابطة خارج المستوي وهذه الإزاحة تزداد بزيادة نسبة الغش.

وهكذا تتضح مدي دقة وسهولة وسرعة تحليل الطيف في منطقة الأشعة تحت الحمراء في كشف الغش في زيت الزيتون البكر الممتاز.